Animal Model for Thoracoscopic Laser Ablation of Emphysematous Pulmonary Bullae

Matthew Brenner, MD, Theodore Shankel, MD, Teri A. Waite, Ann Hamilton, AHT, Diane Bendsza, AHT, Nai-San Wang, MD, PhD, Thomas Milner, PhD, Werner Roeck, PhD, Yona Tadir, MD, Bruce Tromberg, PhD, Archie F. Wilson, MD, PhD, and Michael W. Berns, PhD

Pulmonary and Critical Care Medicine Division, Department of Medicine, and Pathology Department, University of California Irvine Medical Center, Orange, California 92668 (M.B., T.S., N.-S.W., A.F.W.); Beckman Laser Institute and Medical Clinic, Irvine, California 92715 (T.A.W., A.H., W.R., D.B., Y.T., T.M., B.T., M.W.B.)

Background and Objective: Thoracoscopic laser techniques have been described for treatment of pulmonary bullae. Clinical application of this procedure has proliferated despite limited data regarding efficacy or optimal techniques. The objective of this study was to develop an animal model for investigating laser treatment of bullous lung disease.
Study Design/Materials and Methods: Sixty-two New Zealand White rabbits (3–5 kg) were injected intravenously with 0.35 cc sterile-filtered Sephadex G-100 beads (1 g/100 cc suspension). Three hours later, rabbits were anesthetized, intubated, and 10 cc 0.7% heat-treated or 1% untreated carrageenan solution was instilled endotracheally into a catheter wedged in a mainstem bronchus.
Results: Bullae formed over 4–6 weeks in 33% of the animals treated with 0.7% heat-treated carrageenan, and 90% of animals receiving 1% untreated carrageenan ($P < 0.005$) as demonstrated by serial thoracoscopy. Thoracoscopy was performed at 6–8 weeks using 5 mm trocars under general anesthesia and mechanical ventilatory support. Animals developed pulmonary bullae ranging in size from 0.5 to 2 cm. Bullae were ablated under thoracoscopic visualization using a CO_2 laser with a 4 mm OD rigid probe and short focal length in a defocused mode, or an Nd:YAG laser with a 0.4 mm diameter flexible fiberoptic probe. Animals recovered quickly following thoracoscopy.
Conclusion: We have successfully developed an animal model for thoracoscopic laser ablation of emphysematous pulmonary bullae. This animal model should be useful in investigating treatment of bullous lung disease in humans.

Key words: bullectomy, lung, rabbit, thoracoscopy

INTRODUCTION

Emphysema is a condition characterized by pulmonary parenchymal breakdown, leading to formation of holes, or "bullae," in lung tissue. Bullae can expand causing compromise of respiratory function in some patients. Surgical excision of bullae improves pulmonary function in a rare subset of patients in whom isolated bullae become so massive that surrounding pulmonary structures become crowded or "compressed" [1–4]. However, the vast majority of patients have multiple smaller bullae associated with diffuse em-

Accepted for publication November 28, 1994.

Address reprint requests to Dr. Matthew Brenner, Pulmonary and Critical Care Medicine Division, Dept. of Medicine, University of California Irvine Medical Center, Orange, CA 92668.

Dr. Berns did not participate in the editorial review of this paper.

physema. Surgical bullectomy is generally considered to be ineffective in such cases [5–10]. To overcome limitations of surgical bullectomy, we recently developed a thoracoscopic method for laser ablation of emphysematous pulmonary bullae [11,12]. Thoracoscopy is less invasive than thoracotomy or sternotomy, and multiple bullae can be ablated using lasers [11,12], with potential to benefit a larger number of patients with emphysematous pulmonary bullae.

More than 900 patients have been treated with thoracoscopic laser bullae ablation over the past 2 years [11]. Considerable controversy surrounds this procedure regarding a lack of objective proof of efficacy, duration of response, selection criteria, and cost effectiveness. An animal model is needed to investigate many aspects of this procedure and other recently described surgical methods for treatment of emphysematous lung diseases [12].

We describe the successful development of a rabbit model of bullous lung disease and thoracoscopic laser administration techniques. This model should be valuable for investigating laser treatment of bullous lung disease, laser-induced lung injury, lung volume reduction surgery, and for use in clinical training.

MATERIALS AND METHODS

Induction of Emphysematous Bullae

The induction of bullae involves instillation of carrageenan into the airways following intravenous injection of Sephadex beads. We modified methods described by Mitsuhashi et al. [2] to induce bullae as follows.

Prophylactic antibiotic, Baytril 0.22 mg/kg IM, is administered preinduction. Male albino rabbits (3–4 kg) are injected with 0.35 ml of 10 mg/ml Sephadex G-50 beads (100–300 μm diameter, Pharmacia, Uppsala, Sweden) suspended in physiologic saline via marginal ear vein.

Three hours after injection of Sephadex beads, rabbits are anesthetized using inhaled Isoflurane 5%, followed by 0.2cc Ketamine/Xylazine in a 1:1 mixture via intravenous injection. Rabbits are intubated with a 25 gauge guidewire under direct laryngoscopic visualization using a #1 straight blade laryngoscope and placed in a right lateral decubitus position. A 12 gauge, 12″ catheter is inserted over the guidewire into the trachea and passed until wedged. In the 31 treated animals, a rightsided directional guidewire was used to increase likelihood of right mainstem intubation. The catheter is then withdrawn 1–2 cm. Ten milliliters of heat sterilized (15 min., steam autoclave treated 250°F, 15 psi) 0.75% carrageenan (lambda carrageenan #4, Sigma Chemical, St. Louis, MO) solution in physiologic saline are injected into the wedged bronchial catheter. In the 11 most recently treated animals, 10 ml of nonheated 1.0% carrageenan solution in physiologic saline is instilled. The catheter is removed and the rabbit monitored for adequate respiratory function.

Anatomic thoracoscopy is performed 3–4 weeks after induction of emphysematous bullae.

Thoracoscopy

Anesthesia is induced in the rabbits with 2:1 Ketamine HCl (100 mg/ml):Xylazine (20 mg/ml) at a dose of 0.75 cc/kg IM. Animals are intubated with a 3.0–3.5 mm noncuffed endotracheal tube. Oxygen saturation (Ohmeda Biox 3700 Pulse Oximeter, BOC Health Care), end tidal CO2 (Ohmeda 5200 CO2 Monitor, BOC Health Care), and EKG (Hewlett Packard 78353B Continuous EKG Temperature Probe Monitor, BioMedical Services) are monitored continuously. Rabbits are shaved, sterilely prepped with Nolvasan scrub, draped, and placed on ventilatory support using a Harvard Ventilator (Harvard Apparatus Dual Phase Control Respiratory Pump-Canine, Harvard Co., South Natic, MA).

Operative Procedures

Thoracoscopies are performed by strictly sterile surgical procedures. A three trocar approach is used. The initial trocar is placed in the 5th or 6th intercostal space between the pectoralis and *latissimus dorsi* muscles. Full anatomic examination is easily performed from this position. The second trocar is then placed under direct visualization caudally, above the diaphragmatic insertion site. From this position a panoramic view of the thorax is obtained cranially, and the undersurface of the lower lobe could be clearly visualized. A third trocar is placed more medially, usually in the 7th–9th intercostal space, for manipulating instruments or laser probes (Fig. 1).

Thoracoscopy

Thoracoscopy is performed with continuous video assist using an endocamera (Storz Endocam NTSC 202101 20, Karl Stortz, Culver City, CA) and 4 mm rigid 30° modified thoracoscope (Storz Hopkins II 27005B 30°, 4 mm Hysteroscope, Karl Stortz) with a Xenon light source (Stortz Xenon

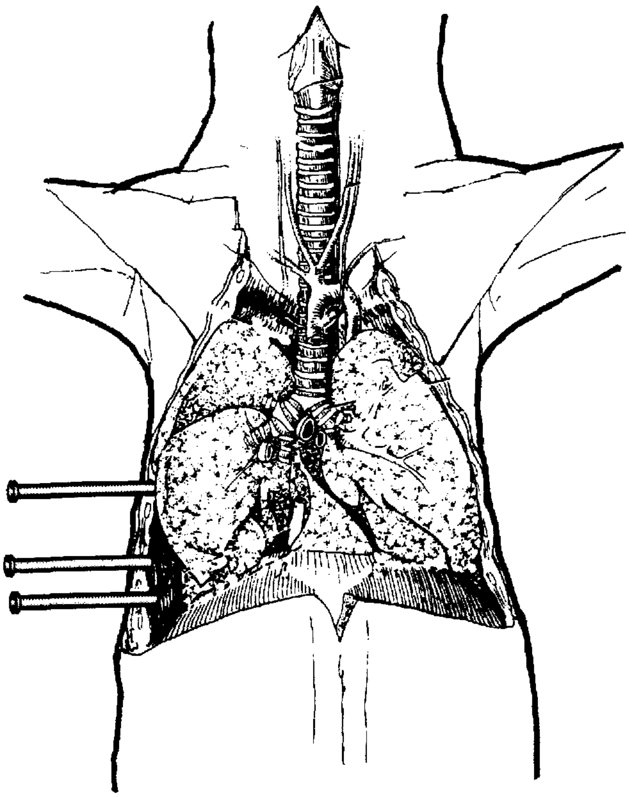

Fig. 1. Location of three thoracic trocars for anatomic examination and laser treatment of emphysematous pulmonary bullae in rabbits.

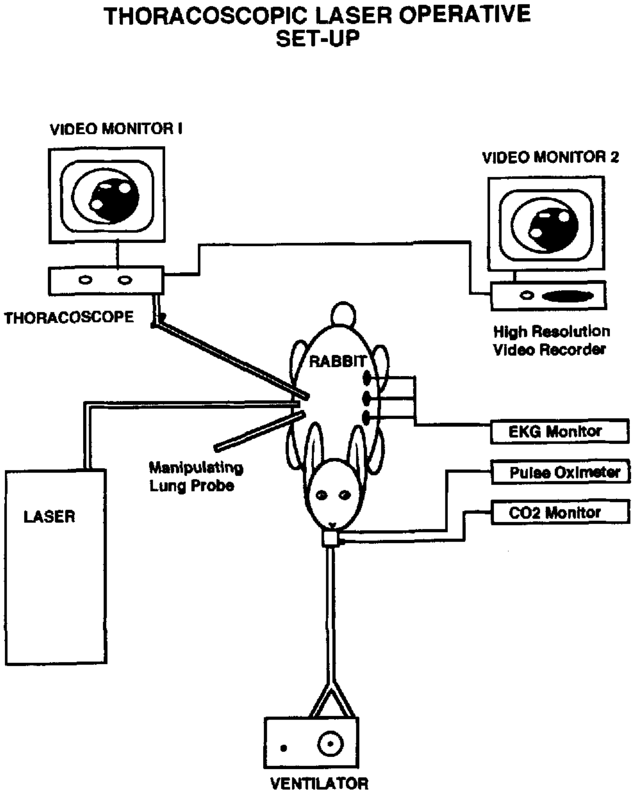

Fig. 2. Set-up for thoracoscopic laser treatment of emphysematous pulmonary bullae.

Light Source 611, Karl Stortz). A high resolution video recorder (Panasonic AG-670P) is connected to two video monitors (Mitsubishi CS-20EX1, Cypress, CA) at opposite ends of the operating table to provide adequate viewing angles for all operating personnel (Fig. 2).

Laser Treatment

We tested the feasability of two different laser delivery modes, a flexible fiberoptic and a rigid delivery system, in this model. A 0.4 mm core diameter plastic-clad silica multimode optical fiber (Endostat 0.4 mm × 12 ft, #0010-0622, San Jose, CA) with a flat cut end is used for Nd:YAG administration (Laserscope KTP laser, operating at 1,064 nm, Laserscope Surgical Laser Systems, San Jose, CA) in a free beam mode. Fiber delivery is calibrated daily prior to use. In this study we used 5 W delivered power with a 3-mm spot size at the lung surface (~8 mm from the fiber tip), which results in a power density of ~70 W/cm^2. The fiber is inserted through the trocar and manually manipulated under video guidance at a distance to 8–10 mm.

A rigid delivery system was used with the CO_2 laser (10,600 nm, Sharplan 1055 CO_2 Surgical Laser System, Laser Industries, Allendale, NJ) through a 4 mm diameter hollow wave guide with a 10 mm focal distance lens at the distal tip (Sharplan CO_2 Laser Laparoscopic Probe, Sharplan 792B F12 lens) [13]. The CO_2 laser is recessed into the trocar 12 mm so that the focal point is 2 mm within the trocar. This enables near maximal attainable spot size within the confines of the thorax.

Rabbits are disconnected from the ventilator during laser exposure in order to prevent lung movement and to maintain a relatively constant distance between the laser tip and target surface. When a CO_2 laser is used, the inspired oxygen concentration is reduced to 21% during laser exposures to avoid risk of combustion.

At the end of the procedure, a 12 Fr neonatal chest tube is placed in the site of one trocar hole. Chest tube position is confirmed with thoracoscopic visualization. The tube is secured percutaneously with 2-0 silk and attached to a Heimlich Chest Valve with suction (Gomco 300, Allied HC, Baxter Hospital Supply, Irvine, CA). If no air leak

is seen following reexpansion, the chest tube is removed.

Histologic Preparation

Animals are anesthetized as previously described, and 1,000 units of heparin are injected intravenously. Two cc Eutha 6 are administered IV just as the descending aorta is severed for exsanguination. The lungs and heart are removed en bloc. Following necropsy, the lung is inflated by intratracheal instillation of 4% formaldehyde in phosphate-buffered solution at 25 cm water pressure for at least 24 hours. Appropriate section are processed routinely, embedded in paraffin, stained with hematoxylin and eosin (H & E), and studied by light microscopy.

RESULTS

Induction of Pulmonary Bullae

Rabbit intubation with a large bore endotracheal catheter was successfully performed in all cases without difficulty. After refinements of our method, 87% of 62 animals survived the initial bullae induction procedure. Two animals developed delayed pneumothorax as bullae were forming. They were managed with chest tube insertion and Heimlich valve venting. One animal died from late pneumothorax (3 weeks postinduction). Radiographs or preliminary anatomic thoracoscopy at 3–4 weeks postinduction documented bullae formation. Thoracoscopically visable bullae formed in 33% of rabbits induced with 0.75% heat-treated carrageenan. In the animals induced using a directional guidewire, the percentage of animals developing increased slightly to 37%. In animals induced with unheated 1% carrageenan ($n = 11$) and a directional catheter, large bullae formed in 90% of the 10 surviving animals ($P < 0.005$ increase in yeild compared to heat-treated carrageenan groups). Bullae ranged in size up to 2 cm in diameter. Bullae formed progressively from areas of pulmonary infiltration over 4–8 weeks as demonstrated thoracoscopically (Fig. 3). Most animals who developed bullae had multiple bullae. Three animals developed diffuse emphysma of at least one lobe without obvious bullae in the heat-treated carrageenan group, and one animal in the unheated carrageenan group.

Thoracoscopy was easily performed in all animals. Visualization of all lobes, including undersurfaces, was obtainable in all. Diaphragm, heart, apex, and parietal pleural surfaces were also well seen.

The three trocar method provided access to necessary regions of the lung for simultaneous use of camera, laser, and manipulating probes. Once the operative lung was collapsed by briefly removing the rabbit from the ventilator, there was no difficulty visualizing the lungs and little or no tendency for the lungs to reexpand during the remainder of the procedure. Image quality using this setup was excellent during our studies (Fig. 3). Visualization was equivalent or superior to open thoracotomy. The procedure is minimally traumatic. Animals were usually ambulating within 15 minutes of completion of the procedure.

On histologic evaluation at 4–5 weeks following induction, the segment of lung instilled with carrageenan was grossly consolidated and microscopically showed thickened alveolar walls with intraalveolar collections of macrophages. Within the consolidated lung, focal regions of alveoli disappeared, producing cystic dilitation of the air spaces. Loose connective tissue and cells often remained in some of the cystic spaces. The subpleural cystic space tended to bulge out and form bullae. Following laser exposure, the bullae appeared shrunken and the cystic space filled by red blood cells, exudates, and a mixture of acute and chronic inflammatory cells. The subpleural alveoli adjacent to the bullae also showed marked congestion and edema.

DISCUSSION

While some studies have proposed thoracoscopic laser therapy may represent a significant advance in the treatment of bullous emphysema [11–13], many questions have arisen regarding this controversial treatment modality. Nonetheless, thoracoscopic laser ablation of emphysematous pulmonary bullae is rapidly being incorporated into clinical practice [11,14]. More than 900 patients have been treated in the past 3 years with reported mortality rates 4–15% [11–13,18] and costs estimated at $24 million. An animal model such as described here is needed to understand the effects of laser treatments or other surgical bullae removal procedures on lungs and to improve techniques.

A rabbit model is ideal because of adequate size, availability, costs, and animal maintenance requirements. Potential problems with rabbit models are the limited space for trocar insertions, laser beam divergence, and limited camera angles available for complete visualization.

We modified the pulmonary bullae induction procedures of Mitsuhashi et al. [2] in order to create a minimally invasive model. By intubating rabbits over a guidewire with a long, large bore flexible endotracheal catheter that could be

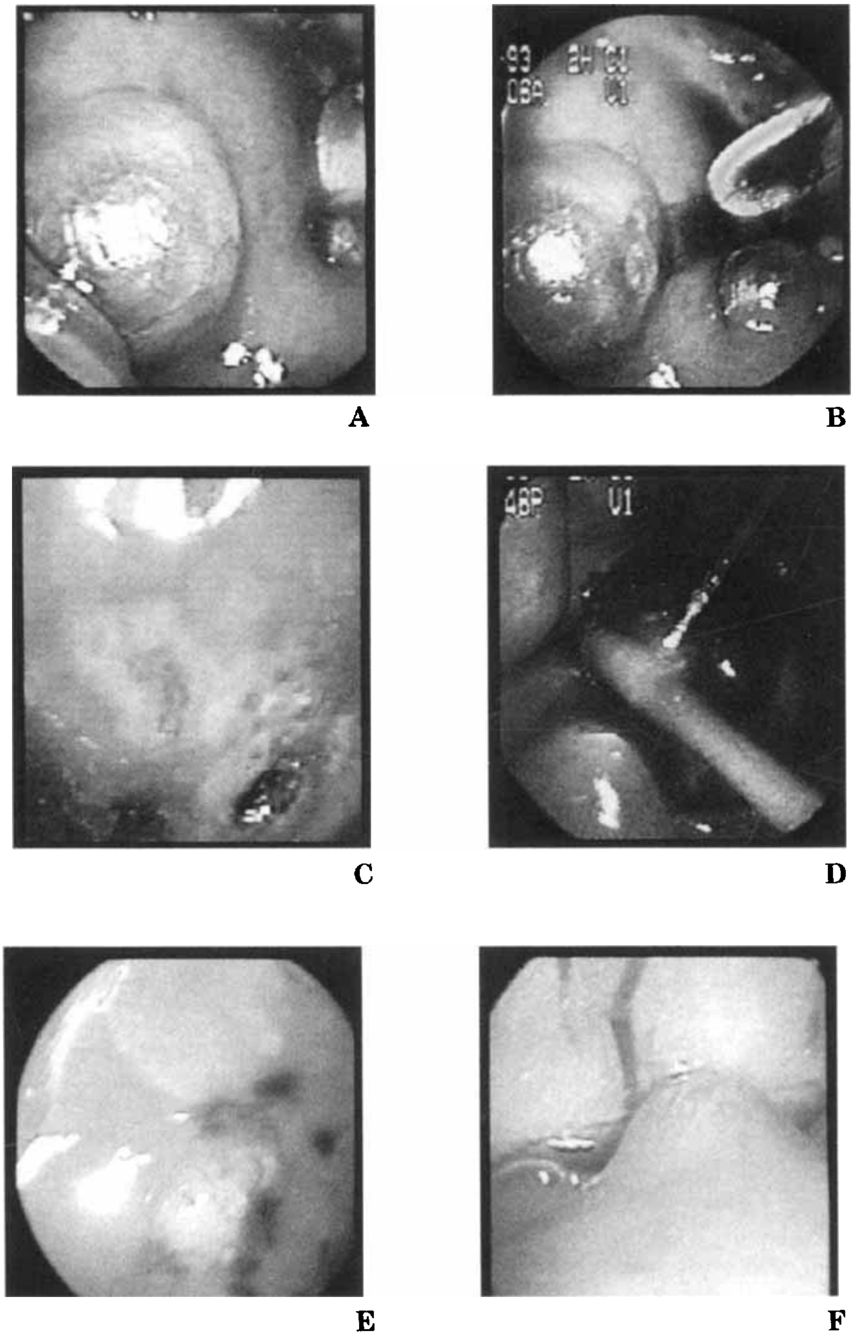

Fig. 3. **A.** Large 1/2–1 cm mature bullae 6 weeks postinduction. **B.** Bulla being treated thoracoscopically with CO2 laser. Note small are of coagulation starting to appear on the lateral surface of the large bulla. **C.** Appearance of the bullae after CO_2 laser treatment. **D.** Nd:YAG 0.4 mm fiber being used to ablate pulmonary bulla in the lower portion of the photograph. A Q-tip is being used to retract the right middle lobe. **E.** Thoracoscopic examinations demonstrating development of pulmonary bullae in a rabbit. The undersurface of the right lower lobe of the same animal seen at 2 weeks postinduction. Note areas of hemorrhage and infarction adjacent to an area of early bulla formation. **F.** Four weeks following induction the bullae are mature and thin-walled as seen from above.

wedged into a mainstem bronchus, we were able to avoid tracheotomy. Carrageenan was instilled in the mainstem bronchus or bronchus intermedius of the dependent right lung. These techniques help to minimize complications. Eight of 62 animals (13%) died during induction in our study compared to 4 of 12 rabbits (33%) receiving comparable dose Sephadex beads reported by Mitsuhashi [2].

Bullae large enough to be evident at thora-

coscopy form in ~1/3 of the animals in our series that received heat-sterilized carrageenan solution. This compares to 60–70% reported by Mitsuhashi et al. [2] who defined bullae histologically (any airspaces >0.31 cm), including bullae located deep within the lung parenchyma. Directing the catheter to the right side increased bullae formation yield only slightly. Increasing the carrageenan concentration to 1% and instilling nonheated carrageenan solution increased the yield of bullae dramatically to >90%. Bullae in these cases were frequently very extensive. Thus heating the carrageenan appeared to have inactivated some component of the carrageenan responsible for bullae formation.

Rogers et al. [15] report difficulty with thoracoscopic visualization in infants using bilateral positive pressure ventilation due to continued inflation of the operative lung. This problem was overcome in the rabbit by inserting an open trocar on the operative side and disconnecting the animal from the ventilator for 10–15 seconds, with resultant collapse of the operative lung. In this manner, thoracoscopy was performed with excellent visualization in all animals despite use of bilateral lung ventilation. For the purposes of this study, we generally preferred three separate trocar sites (Fig. 1). The quality and extent of thoracic visualization were comparable to that seen in adult humans. This procedure is quite simple and can be performed repeatedly without significant complications in the animals.

The narrow confines of the rabbit thorax limit laser defocusing distances. For the CO_2 laser we demonstrated the feasibility of using a hollow wave guide with a divergent lens in the rabbit. Recessing the laser probe a predetermined distance into the trocar such that the focal point was within the trocar enabled maximal defocusing to occur within the limited distance from the end of the trocar. Effective defocusing of the Nd:YAG fiber resulted from wide beam divergence exiting the fiber. In all cases, we were able to obtain spot size and power density comparable to those used in human procedures. Extensive studies will be needed in the future to determine optimal laser techniques, efficacy of therapy, and duration of response.

Thus we report the first description of thoracoscopic methods for creating, examining, and treating pulmonary bullae in a rabbit model. This model should be useful in studying the efficacy and limitations of thoracoscopic laser ablation or volume reduction surgery in bullous lung disease.

ACKNOWLEDGMENTS

This work was supported by DOE Grant #DE-FG03-91ER61227 and ONR Grant #N00014-91-C-0134.

REFERENCES

1. Potgieter PD, Benatar SR, Hewitson RP, Ferguson AD. Surgical treatment of bullous lung disease. Thorax 1981; 36(12):885–890.
2. Mitsuhashi T, Shimazaki M, Chanoki Y, Kuwahara H, Masuda H, Sakai T. Giant to small emphysematous bullae induced by Sephadex beads and carrageenan. Am J Pathol 1986; 124(2):246–252.
3. Sone S, Okubo, A, Ogura, T. Normal human alveolar macrophages have more ability than blood monocytes to produce cell-associated interleukin-1-alpha. Am J Respir Cell Mol Biol 1989; 1(6):507–515.
4. Cominelli F, Nast CC, Clark BD, Schindler R, Lierena R, Eysselein VE, Thompson RC, Dinarello CA. Interleukin 1 (IL-1) gene expression, synthesis, and effect of specific IL-1 receptor blockade in rabbit immune complex colitis. J Clin Invest 1990; 86(3):972–980.
5. Connolly JE, Wilson A. The current status of surgery for bullous emphysema. J Thorac Cardiovasc Surg 1989; 97(3):351–361.
6. Gaensler EA, Jederlinic PJ, FitzGerald MX. Patient work-up for bullectomy. J Thorac Imaging 1986; 1(2):75–93.
7. Gaensler EA, Cugell DW, Knudson RJ, FitzGerald MX. Surgical management of emphysema. Clin Chest Med 1983; 4(3):443–463.
8. FitzGerald MX, Keelan PJ, Cugell DW, Gaensler EA. Long-term results of surgery for bullous emphysema. J Thorac Cardiovasc Surg 1974; 68(4):566–587.
9. FitzGerald MX, Keelan PJ, Gaensler EA. Surgery for bullous emphysema: Case report. Respiration 1973; 30(2):187–200.
10. Head JM, Head LR, Hudson TR, Head JR. The surgical treatment of emphysematous blebs and localized vesicular and bullous emphysema: Analysis of 50 cases. J Thoracic Cardiovasc Surg 1960; 40:443.
11. Wakabayashi A. Thoracoscopic technique for management of giant bullous lung disease. Ann Thorac Surg 1993; 56(3):708–712.
12. Cooper J, Trulock E, Patterson G, Triantafillou A, Sundaresan R, Dresler C, Roper C. Bilateral pneumectomy (volume reduction) for chronic obstructive pulmonary disease. AATS 74th Annual Meeting, 1994, pp 94–95 (abstract).
13. Tadir Y, Karni Z, Fisch B, Rosenberg C, Ovadia J. Tissue effects of a new laparoscopic carbon dioxide laser probe. Fertil Steril 1989; 51(6):1046–1049.
14. Wakabayashi A, Peters H, Singh N, Bennett G, Barnes G, Fujita R, Calmese J. Thoracoscopic treatment of bullous emphysema using sapphire contact tip neodymium yttrium aluminim garnet laser (contact YAG): Preliminary report. Program: 73Rd Annual Meeting of the American Association of Thoracic Surgery Chicago, 1993, Section 47, p 156.
15. Rogers DA, Philippe PG, Lobe TE, Kay GA, Gilchrist BF, Schropp KP, Rao BN. Thoracoscopy in children: An initial experience with an evolving technique. J Laparoendosc Surg 1992; 2(1):7–14.